

Serial No. 10/021,425

3196/00/US (26648)

Amendment and response to Office Action dated April 6, 2004 (Amendment B)

June 29, 2004

### **LISTING OF CLAIMS**

The following listing of claims will replace all prior versions and listings of claims in the present application.

Claim 1. (canceled)

Claim 2. (currently amended) An isolated and purified nucleic acid ~~of claim 1~~ encoding an 11 alpha hydroxylase, wherein said nucleic acid comprises the DNA sequence of SEQ ID NO: 1.

Claims 3-9. (withdrawn)

Claim 10. (currently amended) An isolated and purified nucleic acid encoding an ~~enzyme~~

Aspergillus ochraceus 11 alpha hydroxylase

wherein said hydroxylase that can catalyze the 11 alpha hydroxylation of a compound selected from the group consisting of: comprising 3 keto delta 4,5 steroids (~~3 keto delta 4 steroids~~); ~~3 keto delta 4, 5 delta 6, 7 steroids (3 keto delta 4 delta 6 steroids); 3 keto delta 6, 7 steroids (3 keto delta 6 steroids); and 3 keto delta 1, 2 delta 4, 5 steroids (3 keto delta 1 delta 4 steroids).~~

Claim 11. (canceled)

Claim 12. (currently amended) The isolated and purified nucleic acid of claim 10 ~~or claim 11~~,

wherein said hydroxylation is selected from the group consisting of:

- (a) ~~canrenone to 11 alpha hydroxy canrenone;~~
- (b) ~~(a) androstenedione to 11 alpha hydroxy androstenedione; and~~
- (c) ~~aldona to 11 alpha hydroxy aldonā;~~
- (d) ~~(b) ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD;~~

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- (e) ~~mexrenone to 11 alpha hydroxy mexrenone;~~
- (f) ~~6 beta mexrenone to 11 alpha hydroxy 6 beta mexrenone;~~
- (g) ~~9 alpha mexrenone to 11 alpha hydroxy 9 alpha mexrenone;~~
- (h) ~~12 beta mexrenone to 11 alpha hydroxy 12 beta mexrenone;~~
- (i) ~~delta 12 mexrenone to 11 alpha hydroxy delta 12 mexrenone;~~
- (j) ~~testosterone to 11 alpha hydroxy testosterone;~~
- (k) ~~progesterone to 11 alpha hydroxy progesterone;~~
- (l) ~~mexrenone 6,7 bis lactone to 11 alpha hydroxy mexrenone 6,7 bis-lactone; and~~
- (m) ~~mexrenone 7,9 bislactone to 11 alpha hydroxy mexrenone 7,9-bislactone.~~

Claim 13. (currently amended) The isolated and purified nucleic acid of claim 12, wherein said hydroxylation is selected from the group consisting of: comprises

- (a) ~~canrenone to 11 alpha hydroxy canrenone;~~
- (b) ~~androstenedione to 11 alpha hydroxy androstenedione;~~
- (c) ~~aldona to 11 alpha hydroxy aldon;a; and~~
- (d) ~~ADD (1,4 androstenedienedione) to 11 alpha hydroxy ADD.~~

Claim 14. (canceled)

Claim 15. (currently amended) A method of expressing a protein that can catalyze the 11 alpha hydroxylation of a compound selected form the group consisting of: comprising 3 keto delta 4,5 steroids; ~~3 keto delta 4,5 delta 6,7 steroids; 3 keto delta 6,7 steroids; and 3~~

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~~keto delta 1,2 delta 4,5 steroids~~ further comprising;

- (a) transforming or transfecting host cells with an expression cassette comprising a promoter operably linked to a nucleic acid that encodes said protein, and
- (b) expressing said protein in said host cells.

Claim 16. (currently amended) ~~A-~~ The method of producing the protein of claim 15, further comprising the step of recovering said protein.

Claim 17. (original) The method of claim 15 or claim 16 wherein said protein is an *Aspergillus ochraceus* 11 alpha hydroxylase.

Claim 18. (currently amended) The method of claim 17, further comprising expressing an electron donor protein, wherein said electron donor protein can donate electrons to said protein that can catalyze the 11 alpha hydroxylation of a compound ~~selected from the group consisting of:~~ comprising 3 keto delta 4,5 steroids; 3 keto delta 4,5 delta 6,7 steroids; 3 keto delta 6,7 steroids; and 3 keto delta 1,2 delta 4,5 steroids.

Claim 19. (original) The method of claim 18 wherein said electron donor protein is selected from the group consisting of human oxidoreductase and *Aspergillus ochraceus* oxidoreductase.

Claim 20. (original) The method of claim 18 wherein said electron donor protein is *Aspergillus ochraceus* oxidoreductase.

Claim 21. (original) The method of claim 18, wherein the nucleic acid encoding said 11 alpha hydroxylase and said electron donor protein are on separate expression cassettes.

Claim 22. (currently amended) The method of claim 18, wherein the nucleic acid encoding said 11 alpha hydroxylase and said electron donor protein are on the same expression

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eassettes cassette.

Claim 23. (original) The method of claim 21 wherein said 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is human oxidoreductase.

Claim 24. (original) The method of claim 22 wherein said 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is human oxidoreductase.

Claim 25. (original) The method of claim 21 wherein said 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is *Aspergillus ochraceus* oxidoreductase.

Claim 26. (currently amended) The method of claim 22 wherein said 11 alpha hydroxylase is *Aspergillus ochraceus* 11 alpha hydroxylase and said electron donor protein is *Aspergillus ochraceus* oxidoreductase.

Claim 27. (original) The method of claim 17, wherein said *Aspergillus ochraceus* 11 alpha hydroxylase is SEQ ID NO: 2.

Claim 28. (original) The method of claim 19, wherein said human oxidoreductase is SEQ ID NO: 4.

Claim 29. (original) The method of claim 19, wherein said *Aspergillus ochraceus* oxidoreductase is SEQ ID NO: 6.

Claims 30-34. (withdrawn)

Claim 35. (currently amended) An expression cassette comprising a promoter operably linked to ~~an the~~ isolated and purified nucleic acid of claim 10. ~~encoding a polypeptide that can~~

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~~catalyze the 11 alpha hydroxylation of a compound selected from the group consisting of:~~  
~~3 keto delta 4,5 steroids (3 keto delta 4 steroids); 3 keto delta 4,5 delta 6,7 steroids (3~~  
~~keto delta 4 delta 6 steroids); 3 keto delta 6,7 steroids (3 keto delta 6 steroids); and 3~~  
~~keto delta 1,2 delta 4,5 steroids (3 keto delta 1 delta 4 steroids).~~

Claims 36-45. (withdrawn)

Claim 46. (currently amended) ~~A-~~ The host cell harboring an expression cassette of any of  
claim 35.

Claim 47. (currently amended) ~~A-~~ The host cell of claim 46, wherein said expression cassette is  
integrated into the chromosome of said host cell.

Claim 48. (currently amended) ~~A-~~ The host cell of claim 46, wherein said expression cassette  
is integrated into an expression vector.

Claim 49. (currently amended) A method of determining the specific activity of a cloned 11  
alpha hydroxylase comprising the steps of;

- (a) transforming host cells with an expression vector comprising a nucleic acid that  
encodes said ~~11 alpha~~ hydroxylase,  
wherein said hydroxylase can catalyze the 11 alpha hydroxylation of a compound  
comprising 3 keto delta 4,5 steroids (3 keto delta 4 steroids);
- (b) expressing said ~~11 alpha~~ hydroxylase in said host cells;
- (c) preparing subcellular membrane ~~fractions~~ fraction microsomes from said cells,
- (d) incubating said subcellular membrane ~~fractions~~ fraction microsomes with a  
steroid substrate, and
- (e) monitoring conversion of the steroid substrate to its 11 alpha hydroxy steroid

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counterpart.

Claims 50-56. (withdrawn)

Claim 57. (canceled)

Claim 58. (original) ~~An~~ The isolated nucleic acid that specifically hybridizes under highly stringent conditions to the nucleic acid of claim 2.

Claims 59-77. (withdrawn)

Claim 78. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide is at least 99% identical to SEQ ID NO:2.

Claim 79. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide is at least 95% identical to SEQ ID NO:2.

Claim 80. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide is at least 90% identical to SEQ ID NO:2.

Claim 81. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide is at least 75% identical to SEQ ID NO:2.

Claim 82. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide is at least 50% identical to SEQ ID NO:2.

Claim 83. (new) An isolated and purified nucleic acid encoding a polypeptide, wherein said polypeptide comprises at least one conservative amino acid residue substitution of SEQ ID NO:2.